Stability Report

TRI-50c-00-STAB-01-Report

for

Trigen Ltd.

Dr. D. Krimmer

Active Ingredient stability data of TRI-50c-00

Status:

Pilot Scale batches

Preclinical Trials

Approval of Stability Report

The undersigned herewith confirm that the present study was performed by us or under our supervision in accordance with the methods described. This report is checked for accuracy, completeness and compliance with the ruling guidances. This stability study was performed according to the approved stability protocol TRI-50c-00-STAB-01.

Dr. A. Weiland, 2.7.04

Dr. A. Weiland

(Project Leader)

E. J. Maier 02/07/04

E. J. Maier

(Study Director)

Content

1 ORGANIZATION 4

2 OBJECTIVES 4

2.1 Additional Information 5

3 SPECIFICATION 5

4 RESULTS 6

4.1 Appearance 7

4.2 Assay 7

4.3 Degradation Profile 7

4.4 Moisture 9

4.5 Residual Solvents 9

4.6 Further Tests 9

5 DISCUSSION 9

6 CONCLUSIONS 10

7 TABLES 11

7.1 Table 1: Appearance 11

7.2 Table 2: Moisture Content by Karl Fischer Titration 11

7.3 Table 3: Impurity Profile 12

7.4 Table 4: Residual Solvents 13

8 REFERENCES 14

1 Organization

This stability study was performed by the company LPU. It was the overall responsibility of the company LPU to carry out the study according to the procedures described in the stability protocol TRI-50c-00-STAB-01.

Sponsor	**Trigen**
Address	Trigen Ltd
	Clareville House
	26-27 Oxendon Street
	London SW1 4EL
Phone	+44 20 70042630
Fax	+44 20 7004 2631
Contact person	Dr. A. Weiland/ Dr. D. Krimmer

Contractor	**LPU**
Address	LPU Labor fuer Pharma- und Umweltanalytik
	Fraunhofer Strasse 11a
	82152 Planegg / Martinsried
Phone	+49 89 8992290
Fax	+49 89 8577899
Contact person	Dr. W. Hartwich

2 Objectives

The stability of the pilot scale batch TRI50c-00-0210007 was investigated according to the ICH guidelines. In the stability protocol TRI-50c-00-STAB-01 the selected batch for this study is presented.

2.1 Additional Information

For information regarding test methodology, test parameters, storage conditions, manufacturing information, batch information and packaging information please refer to the stability protocol TRI-50c-00-STAB-01.

3 Specification

Tab. 3.1: Specifications (Test parameter, method, acceptance limit)

Testparameter	Method	Method - ID Version/Date	Preliminary acceptance limit	Remark
Appearance	Visual	N/A	White to off white powder	
Colour	Visual	N/A	Off-white	
Water Content	Karl Fischer Titration	USP	< 5%	not applicable
Enantiomeric Purity HPLC	NP-HPLC[2)]	NP1 (from 02.03.2003)	< 0.5% of unwanted isomer	to be developed
Assay by HPLC	RP-HPLC[1)]	RP1 (from 02.03.2003, later SOP 09-01-163)	98% - 102%	
Assay by titration	Potentiometrically[2)]	N/A	> 98%	not applicable
Residual solvents	GC ($CHCl_3$)[3)]			
Identity	Boron-Ethyl ester, IR, chemical ion detection[2)]	Boron (later SOP 09-17-009.R00) IR (later SOP 09-07-009.R00) Chem.Ion Det. N/A		identity of the acid and absence of related salt ions
Impurity I	RP-LC[1)]	RP1 (from 02.03.2003, later SOP 09-01-163)	< 0.5%	

Impurity II	RP-LC[1)]	RP1 (from 02.03.2003, later SOP 09-01-163)	< 0.5%	
Impurity III	RP-LC[1)]	RP1 (from 02.03.2003, later SOP 09-01-163)	< 0.5%	
Benzyl alcohol	RP-LC[1)]	RP1 (from 02.03.2003, later SOP 09-01-163)	< 0.25%	
Benzoic acid	RP-LC[1)]	RP1 (from 02.03.2003, later SOP 09-01-163)	< 0.25%	
Benzaldehyde	RP-LC[1)]	RP1 (from 02.03.2003, later SOP 09-01-163)	< 0.25%	
Unknown (quantified as TRI50c-00)	RP-LC[1)]	RP1 (from 02.03.2003, later SOP 09-01-163)	< 0.25%	

1) partly validated 2) to be established 3) developed

4 Results

Only data up to 3 months storage will be reported here. After 3 months the material had largely decomposed, so that the stability protocol was stopped. There were so many peaks present, that it was difficult to get all chromatograms integrated the same way. After discussion it was decided only to assess the major impurities/degradation products in this report: Impurity I, Benzyl Alcohol, Benzoic Acid, Benzaldehyde, unknown 1 (RRT 0.93), unknown 6 (RRT 0.95) and unknown 7 (RRT 1.07).

To gain further supportive information about the decomposition of the material, the material was tested after 6 months and the results were used for the qualification of the unknown impurities (decision: analytical meeting 27Feb2004 at LPU). The first chromatographic result was not satisfactory, which was put down to the deterioration of the HPLC column. Therefore one of the t=6, 40°C/75% rh samples was retested using a newer column (X-Terra column of the same lot). It could be shown that the separation of impurities on the rerun sample has improved significantly.

4.1 Appearance

See table 1
No significant change in the appearance of the API has been observed after storage for 1 month at 25℃/60%r.h.. After 3 months at 25℃/60% r.h, however, the appearance of the sample was noted to be darker in comparison to the samples after 1 month.

After 1 month storage at 40℃/75% r.h, the appearance of the API had changed from white to dark brown.

4.2 Assay

See table 3.
Even for samples stored at 25℃ and 60% r.h., the assay failed specification (98-102 %w/w) after only 1 month.

4.3 Degradation Profile

See table 3.
From the start of the stability study there were impurities present.

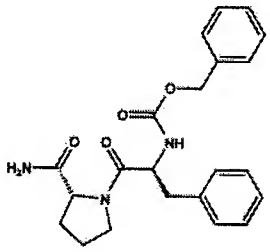
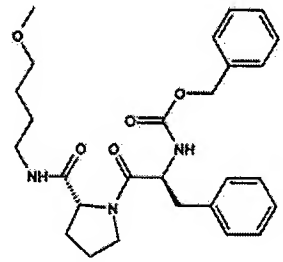
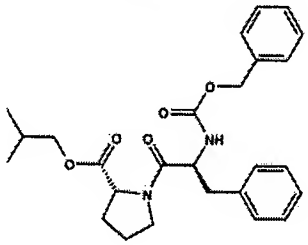
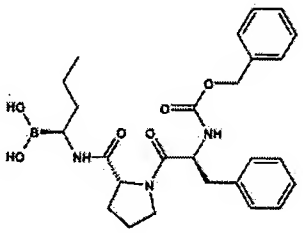
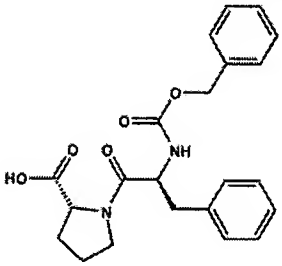
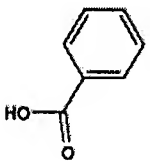
From 1 month into the study all samples as well at 25℃/60% r. h, 30℃/70% r. h as at 40℃/75% r. h had a level of Impurity I of larger than 2.5% w/w. This impurity increased at the 25℃ (60% r. h) conditions to about 13% after 3 months. For the conditions 40℃ (75% r. h), impurity I increased from almost 11 % w/w at 1 month to almost 24% w/w at 3 months.

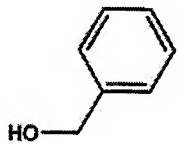
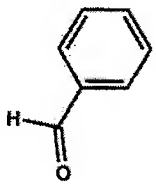
Significant degradation was observed after 3 months storage at 25℃ and 60% r.h. The smallest impurity peak is 0.18% and the largest is 13.32%. The sum of impurities is 33.84%.

For the storage at 30℃ (70% r.h) the sum of impurities after 1 month was 30.95% (6 peaks) and after 3 months 30.56% (6 peaks). The major impurity was impurity I.

For the storage at 40℃ (75% r.h) the sum of impurities after 1 month was 25.09% (7 peaks) and after 3 months 35.64% (6 peaks). The major impurity was impurity I.

4.3.1 Identification of Degradation Products

Impurity I:	Impurity II:
	
Impurity III:	**Impurity IV:**
	
Z-Dipin H (Dipeptide)	**Benzoic acid**
	

Benzylalcohol	Benzaldehyde
	

4.4 Moisture

See table 2.
The moisture content of the API was determined by Karl Fischer Titration. As time and experience showed, this means of water determination was not suitable for this substance. The boronic acid group reacts with the alcohol in the reagent and forms an ester group. This results in the generation of water which then is titrated with the reagent. Therefore the obtained water value is too high and the true water value is not known.

4.5 Residual Solvents

The t=0 value was 5.68% w/w of chloroform. The determination of the residual solvent chloroform was carried out by GC. As expected, the residual solvent content of the samples is higher at lower temperatures. After 3 months, the chloroform content in the samples is ≤0.05% w/w, except for –20°C samples.

4.6 Further Tests

Some tests that had been required in the specifications were not carried out due to a lack of methods. Even though a lot of time and effort were put into the development of an HPLC method for the determination of the enantiomeric purity of TRI50c-00 and into the development of a potentiometric titration of the API assay, no suitable methods could be established. For proof of identity some methods like HPLC retention time, boron-ethylester flame and IR-spectroscopy were used so far.

5 Discussion

The TRI50c acid has been shown not to be stable when stored at 25°C and 60% r.h for any length of time. The only way to store this substance is to keep it at -20°C. TRI50c-00 showed to be stable at -20°C for 3 months. Therefore, the acid is not suitable for storage.

6 Conclusions

Data show that the free acid is only suitable to be stored at -20°C if to be used e.g. as reference substance for bioanalytical studies.

Lot TRI 50c-00-0210007 has shown to be stable only for ca. 6 months. Limited data on additional exploratory laboratory lots of TRI 50c-00 indicate an acceptable stability of more than 12 months but only if stored under exclusion of moisture and at least -20°C.

It is evident that TRI 50c-00 is not suitable as a key intermediate to be stored prior to production of TRI 50c salts for its stability and also does not appear to be a suitable candidate as API for formulation purpose.

Data from a reference standard quality comparison with an earlier lot of the acid demonstrated that a higher level of purity is possible (please refer to the reference standard report). It is anticipated that a stress stability study with a purer starting material would have shown better results.

7 Tables

7.1 Table 1: Appearance

Stability Condition	Time point		
	Initial	1 month	3 month
-20℃	White	White	White
25℃/60%RH		White	Brown
30℃/70%RH		Yellow/Brown	Dark Brown
40℃/75%RH		Brown	Dark Brown

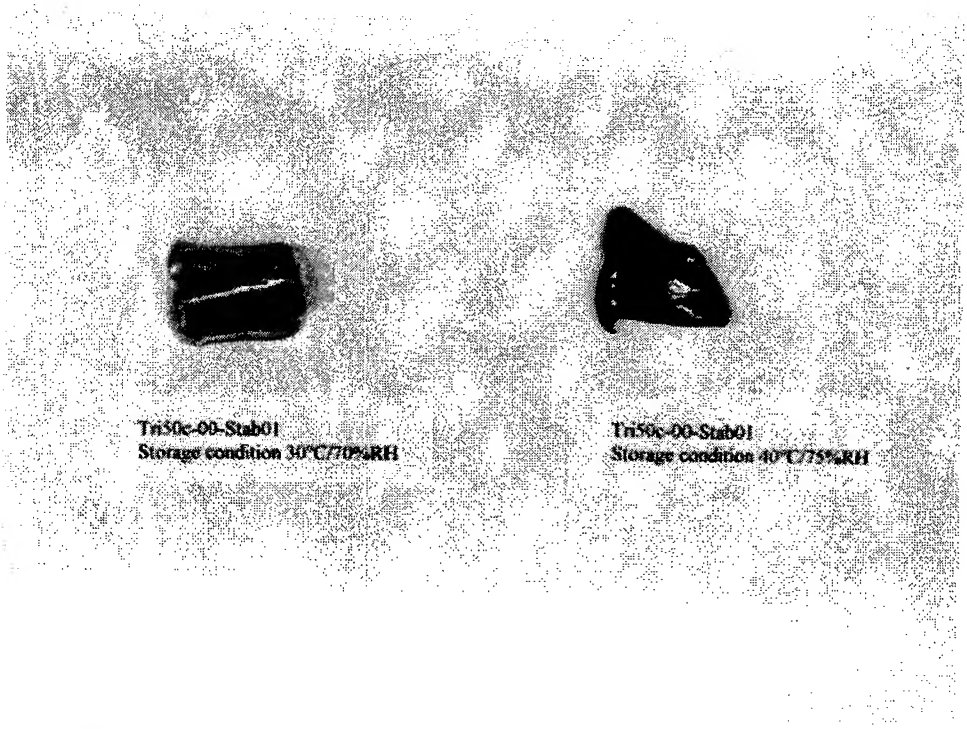


Figure 7.1 Appearance of TRI50c-00 at 30℃/70% r.h. and 40℃/75% r.h. after 1 month

7.2 Table 2: Moisture Content by Karl Fischer Titration

Stability Condition	Time point		
	Initial	1 month	3 month
-20℃	3.96% w/w	3.26% w/w	3.94% w/w
25℃/60%RH		5.45% w/w	7.66%w/w
30℃/70%RH		6.02% w/w	8.77%w/w
40℃/75%RH		6.80% w/w	9.86% w/w

7.3 Table 3: Impurity Profile in %w/w

Temperature			°-20°C	°-20°C		25°C	25°C		30°C	30°C		40°C	40°C
Time [month]		0	1	3		1	3		1	3		1	3
Assay (HPLC) *)		97.18	96.74	99.11		83.17	58.83		67.79	44.39		62.53	43.15
	RRT(RT API= 12.5 min)												
Impurity I) (11,6 min)**	**0.93**	**0.59**	**0.88**	**0.95**		**2.86**	**13.32**		**9.51**	**17.87**		**10.86**	**23.98**
Impurity II **) (13,9 min)	1.11	0.00	0.00	0.00		0.00	0.00		0.00	0.00		0.00	0.00
Impurity III *) (18,4 min)	1.47	0.00	0.00	0.00		0.00	0.00		0.00	0.00		0.00	0.00
Impurity IV *)		0.00	0.00	0.00		0.00	0.00		0.00	0.00		0.00	0.00
Benzylalcohol **) (4,2 min)	**0.34**	**0.00**	**0.00**	**0.00**		**0.00**	**0.00**		**0.00**	**0.00**		**0.00**	**0.00**
Benzoic acid **) (6,0 min)	**0.48**	**0.03**	**0.02**	**0.00**		**0.26**	**0.82**		**0.62**	**0.84**		**0.47**	**0.64**
Benzaldehyde **) (6,6 min)	**0.53**	**0.04**	**0.04**	**0.04**		**0.10**	**0.18**		**0.18**	**0.12**		**0.13**	**0.10**
Unknown I *) (14,9 min)	**1.19**	**5.95**	**5.74**	**5.10**		**3.88**	**3.26**		**5.42**	**1.91**		**2.90**	**1.91**
Unknown II *) (15,1 min)	1.21	0.00	0.00	0.00		0.00	0.00		0.00	0.00		0.00	0.00
Unknown III *) (16,9 min)	0.72	0.00	0.00	0.00		0.00	0.00		0.00	0.00		0.00	0.00
Unknown IV *) (8.6 min)	0.69	0.00	0.00	0.00		0.00	0.00		0.00	0.00		0.00	0.00
Unknown V *) (10,4 min)	0.83	0.00	0.00	0.00		0.00	0.00		0.00	0.00		0.00	0.00
Unknown VI *) (11,9 min)	**0.95**	**0.00**	**0.64**	**0.00**		**1.51**	**6.80**		**5.86**	**7.40**		**4.21**	**3.99**
Unknown VII *) (13,4 min)	**1.07**	**2.84**	**2.79**	**2.11**		**4.58**	**9.46**		**9.36**	**2.42**		**5.48**	**5.02**

*) calculated using TRI50c-00 as reference

**) calculated using the respective impurity as reference

7.4 Table 4: Residual Solvents

Temperature		-20°C	-20°C		25°C	25°C		30°C	30°C		40°C	40°C
Time [month]	0	1	3		1	3		1	3		1	3
Residual Solvents (Chloroform) in %w/w	5.68	4.12	4.94		1.08	0.03		0.33	0.05		0.36	0.05

8 References

- stability protocol TRI-50c-00-STAB-01 (SP_10555_104_0203)
- ICH-Guideline **Q1AR**: Stability Testing of New Drug Substances/Products
- ICH Guideline **Q6A**: Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances
- Report no. A-31050-173-1002/05 Schmitz "Method development and validation of a chromatographic method (HPLC) to assess potency and impurity profile of Tri50c-00"
- Report no. TRI-50c-3.2.P.6/101 by A. Weiland "TRI50c: Reference Standards: LC-MSMS assay for bioanalytical measurements"